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Salmonella genes involved in attachment on carcass surface and pork meat contamination

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BACKGROUND

Human infections caused by Salmonella from contaminated food is a common problem. Consequently, there is a need for optimizing food production environment in order to reduce any threat to human health caused by the food chain.

time-course of the expression profile of attachment, virulence and lag-phase genes to see if specific probes can be designed to be used for "early-warning" assays. This may give us an idea of what kind of food products and production environments that facilitate expression of lag-phase genes.

AIM

Establish a new approach to identify and use genes of Salmonella involved in the attachment on carcass surfaces and in pork chain contamination. With the design of a new meat surface model, we will analyze a number of meat and non-meat isolates for their ability to express the attachment genes. Subsequently, we will look at the

STRATEGY

- Identify genes involved in attachment to meat
- Study the influence of the "history" of Salmonella on attachment potential
- Measure the expression of selected genes over time for Salmonella on a meat surface

METHODS

1. Identify genes involved in attachment to meat

- Genes are selected from literature studies and expression data from gel cassette system (KU-Life)
- Selected genes:
 - flagellar genes (fli, flj, flh, flg, mot)
 - fimbriae and curli genes (agf, fim, pef, lpf, std)
 - genes related to extracellular matrix (prg, bap, mis, shd)

2. Attachment studies – "history of cells"

- Influence of physiological state of Salmonella on adhesion is studied:
 - Liquid state broth
 - Immobilized state gel cassette
- Gel cassette system developed by IFR (Brocklehurst et al., 1995)

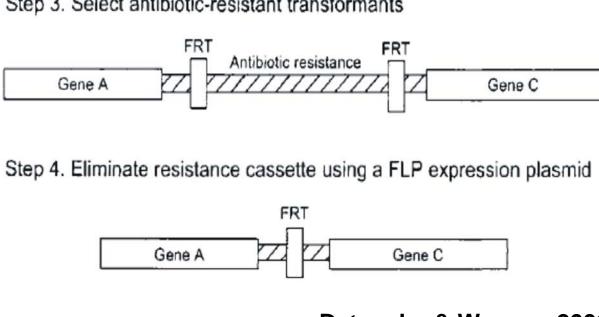


Gel cassette ready for inoculation medium (IFR)

- genes involved in cellulose biosynthesis (*yhj*, *bcs*)
- Knock-out mutants are constructed using lambda red system (Datsenko & Wanner, 2000) and P22 transduction - Strain: S. Typhimurium 4/74
- The ability to attach to meat for mutant cells and wild type cells is compared

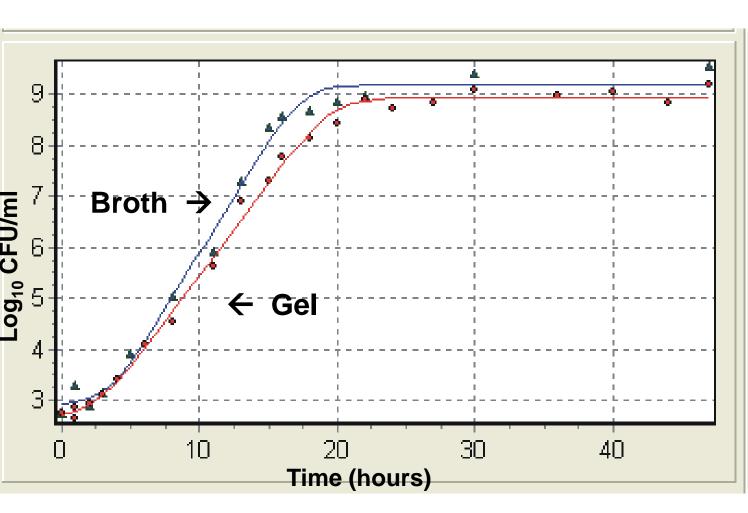
	Antibiotic resistance
ransform strain	expressing λ Red recombinase
ransform strain	expressing λ Red recombinase <u>H2</u>

. PCR amplify FRT-flanked resistance gene



Datsenko & Wanner, 2000

- Growth experiments in broth + gel:
- Temperature: 25 °C
- Medium (gel): LB + 22% pluronic - Medium (broth): LB

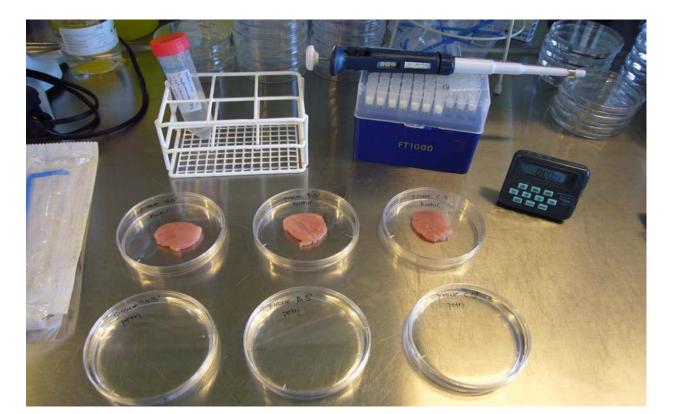


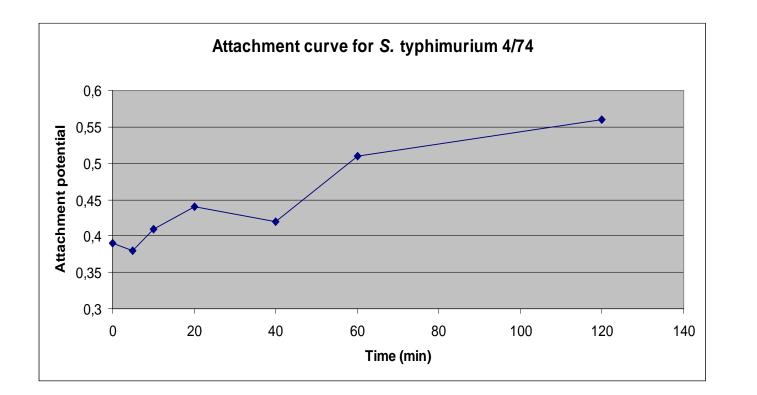
Growth curves for S. Typhimurium 4/74

- Directly from broth and gel cassette cells are applied to a meat model at the same inoculation level (~ 10⁸ CFU/ml)

3. Attachment studies – pork meat surface model

Salmonella cells from broth and gel are diluted ten times in sterilized maximum recovery diluent (MRD) and inoculated onto pieces (9.6 cm²) of pork fillets (meat model)





4. Time-course of gene expression on meat

- Isolate RNA from cells strongly attached to meat over time:
 - Qiagen RNeasy mini kit using combined enzymatic lysis and Proteinase K digestion
 - Quality check using Bioanalyzer

Meat mode

Initial test study of attachment potential of Salmonella to pork meat

Ino	culation level (CFU/ml)	Strain	Adhesion time (min)	Temperature	Treatment	Attachment potential
	~ 10 ⁷) µl spread on heat surface	S. Typhimurium 4/74: wt vs. mutant gel vs. broth	0, 5, 10, 30, 60, 120	Room temp.	Loosly attached: wash in 40 ml MRD for 1 min Strongly attached: stomach in 40 ml MRD for 1 min	Calculated as: <u>Strongly</u> Strongly+Loosly

- Conversion of total RNA into cDNA
- Preparing for expression analysis either by whole genome microarray technology or by quantitative RT-PCR
- Genes of interest: attachment, virulence and lag-phase

- Optimization needed:

- Minimize background flora?
- Increase amount of cells extracted from the meat surface?



Microarray lab at National Food Institute, DTU



REFERENCES

Brocklehurst TF, et al. (1995). JFM 27:45-60. **Datsenko KA & Wanner BL (2000).** PNAS 97:6640-6645.